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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CEFOXITIN SODIUM FOR INJECTION BY UV VISIBLE SPECTROPHOTOMETRY

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ABSTRACT

A rapid, cheap, reliable and simple spectrophotometric method for the quantitive determination of Cefoxitin in powder for injection dosage forms was developed. The proposed method obeys Beer's law limit in the range of 10 – 50 µg/mL with an apparent molar absorptivity of 8.451 x 103 L mol-1 cm-1. Linear regression of absorbance on concentration gave the equation Y= 0.026X – 0.167, with a correlation co-efficient of R2 = 0.999. The detection wavelength was found at 231 nm. LOD and LOQ were found to be 1.39µg/mL and 4.21µg/mL for Cefoxitin sodium respectively. The accuracy of the method was proved by performing recovery studies which were carried out by analyzing the formulation with three different levels like 10%, 20% and 30%. A value closer to 100% indicates that the proposed method is accurate for the analysis. Thus, it was concluded that the above method was simple, precise and easy to perform and require short time to analyze the drug in the commercial formulations.

KEYWORDS: Cefoxitin Sodium, UV, Method development, Validation.

INTRODUCTION

Cefoxitin Sodium is a semi-synthetic, broad-spectrum cepha antibiotic for intravenous administration. It is derived from cephamycin C, which is produced by Streptomyces lactamdurans. The bactericidal action of cefoxitin results from inhibition of cell wall synthesis.[1-4] IUPAC name is (6R,7S)-3-[(carbamoyloxy)methyl]-7-methoxy-8-oxo-7-[2-(thiophen-2-yl)acetamido]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. Molecular formula C16H17N3O7S2Na. Molecular Weight is 427.4.

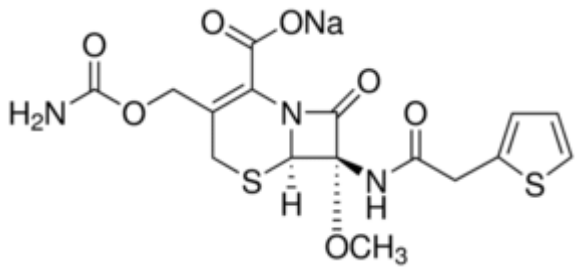

Fig. 1: Structure of Cefoxitin Sodium.

A simple, harmonized approach to HPLC method screening can reduce cycle time for method development. Reducing expenses and improving efficiency have been a focus for many pharmaceutical companies. Several examples in the literature discuss the use of streamlined method development or screening processes. A recent publication by Xiao et al. used the ChromSword® method development software in conjunction with automated column switching for challenging separations (e.g., alpha and beta methylepoxide) while utilizing columns from major vendors4 . Other examples illustrate automation for peak tracking as well as column and mobile phasae screening in addition to the use of software tools for optimization (ChromSword®)[5-6] The RP-HPLC method is widely employed in quality control assessment of drugs because of their sensitivity, repeatability and specificity7-11. On the other hand, the use of spectroscopic techniques can be considered a promising simple, faster, direct and relatively less expensive alternative for the determination of active drug content in pharmaceutical formulations with sufficient reliability.[12-13]

The objective of the present work is to develop & validate stability indicating, simple and accurate method for the determination of Cefoxitin Sodium using accurate UV spectroscopic method for the determination of Cefoxitin sodium in powder for injection and perform validation of the method as per ICH guidelines (International Conference on Harmonization of Technical requirements for registration of pharmaceuticals for human use).

MATERIALS AND METHODS

Instruments

- Digital balance - Model No: US – 500C.
- UV – Visible spectrophotometer- Systronics 2201 Double beam with pair of 10mm matched Quartz cells
- Calibrated glass wares.

Reagents and chemicals

All the chemicals and reagents used were of analytical grade

- Distilled water.
- 0.1M sodium hydroxide - Merck – AR grade

Drug samples

Cefoxitin active pharmaceutical ingredient was obtained from orchid chemicals and pharmaceutical LTD.
Cefoxitin sodium - 99.7 %

Formulation used

The finished product Cefoxitin sodium powder for injection was used which was formulated by Hospira Health care PVT LTD.

METHOD DEVELOPMENT

Preliminary solubility studies of pure drug Cefoxitin sodium

Cefoxitin sodium is very soluble in water; soluble in methanol; sparingly soluble in dimethyl formamide; slightly soluble in acetone; insoluble in ether and in chloroform. The solubility of Cefoxitin sodium was determined by using different aqueous systems like distilled water, 0.1M NaOH. The solvent selected for the UV study was 0.1M NaOH.

Selection of wavelength

The standard solution of Cefoxitin sodium was taken and scanned in the range of 200 – 400 nm in the UV – Visible spectrophotometer. From the spectrum obtained the wavelength at which the maximum absorption takes place has been found out. From the spectrum taken, it was found out that the λ_{max} for Cefoxitin sodium was observed at 231 nm.

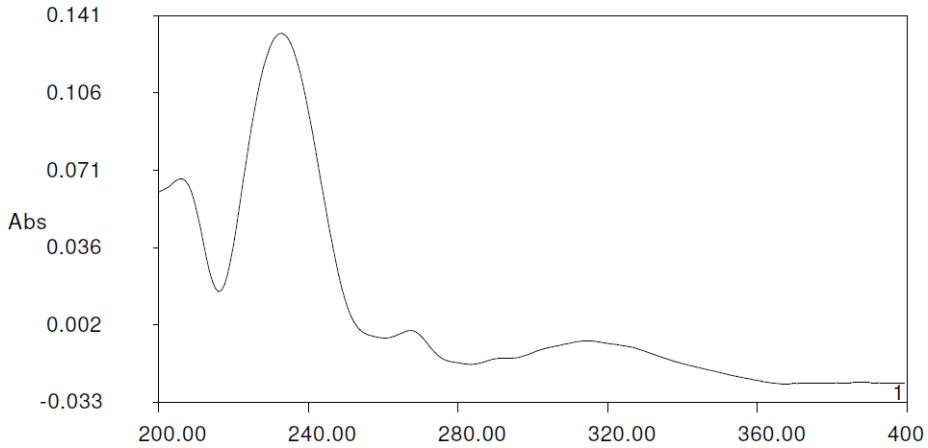

Fig. 2: Spectrum of Cefoxitin sodium scanned between 200 - 400 nm.

Preparation of standard solutions for calibration curve

Accurately weighed 100 mg (1000 µg/ml) of Cefoxitin sodium pure drug was taken in separate 100 ml volumetric flask and diluted with 0.1 M sodium hydroxide solution (standard stock solution). Further dilution was made by pipetting out 10 ml (100 µg/ml) of standard stock solution and by transferring into 100 ml volumetric flask, diluted with 0.1M sodium hydroxide solution. Final dilutions were made by transferring 1, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 ml from the 100 µg/ml solution into the 10 ml volumetric flask and then volume made with 0.1 M sodium hydroxide to obtain 10, 15, 20, 25, 30, 35, 40, 45, 50 µg/ml solutions (10-50 µg/ml). Evaluation was performed with ultra – violet detector for Cefoxitin sodium at 231 nm. Plots of concentration Vs absorbance were prepared.

Formulation used

Cefoxitin sodium for Injection 1gm.

Preparation of sample solution for assay of formulation

One vial was taken and was weighed. Then one vial was reconstituted with 10ml of water for injection or as per labeling to get 95mg/ml of Cefoxitin. Then entire contents was withdrawn from the vial using a suitable calibrated Hamilton syringe & transfered the solution to 250ml volumetric flask and diluted to volume with 0.1 N NaOH and mixed well. 0.5ml of resulting solution was further diluted to 100ml with 0.1 N NaOH to get final concentration of 20 µg/ml Cefoxitin respectively. The absorbance of final solution was measured at 231nm.

METHOD VALIDATION

The method was validated according to ICH, US FDA guidelines and USP. Method validation provides an assurance of reliability during normal use, and is sometime referred to as "The process of providing documented evidence that the method does what it is intended to do".

Assay of Cefoxitin sodium for injection

The absorbance of the sample solution in the concentration of 20µg/ml was measured at 231 nm and percentage drug content was calculated in replicate.

Table 1: Assay of Cefoxitin sodium for injection.

Concentration (µg/mL)	Label claim (mg)	Percentage Content *
20	1000	99.47 %

(*n=3)

Linearity and range

Linearity of the proposed method was verified by analyzing different concentrations in the range of 10-50µg/mL for Cefoxitin sodium. They were established along the standard curve. The regression coefficient, y-intercept and slope of the regression line were calculated for the drug.

Precision – system precision

The *interday* precision of the developed method was evaluated by analyzing aliquots of Cefoxitn sodium sample from homogeneous lot (concentration 20µg/mL) for six times on the same day. The *intrad*ay precision was evaluated from the same concentration on six consecutive days.

Accuracy

The accuracy of the method was performed by conducting the recovery studies at three levels (10, 20 and 30 %). The actual and measured concentrations were then compared. Three concentrations of the drug solution were prepared and analyzed in triplicates. The percentage recovery and % RSD was calculated.

LOD & LOQ

For the determination of LOD and LOQ, the method is based on residual standard deviation of regression line and slope. To determine LOD & LOQ the specific calibration curve was studied using the sample containing analyte in the range of detection limit and quantitation limit.

RESULTS AND DISCUSSION

Solubility

Cefoxitin sodium is very soluble in water, soluble in methanol, sparingly soluble in dimethyl formamide, slightly soluble in acetone, insoluble in ether and in chloroform. The solubility of Cefoxitin sodium was determined by using different aqueous systems like distilled water, 0.1M NaOH, 0.1M HCl. From the above mentioned solubility studies it was found that the drug has the solubility in water and 0.1M NaOH. The solvent selected for this method was 0.1M NaOH, because in water the drug does not show stable λ-Max graph.

Parameters for calibrated curve

The optical characteristics such as absorption maxima, Beer Law limit, molar absorptivity and Sandell's sensitivity of standard drug were calculated from the average of six measurements and results were tabulated on Table 2.

Table 2: Parameters for calibration curve.

S. No	Optimal parameters	Values
1	λ-Max (nm)	231
2	Linearity (µg/mL)	10-50
3	Regression equation	Y= 0.026X – 0.167
4	Correlation coefficient	0.999
5	Slope	0.026
6	Intercept	0.167
7	Molar absorptivity (L $mol^{-1}cm^{-1}$)	8.451 x 10^3
8	Sandell's sensitivity (µg/cm^2/0.001)	0.056
9	LOD (µg/mL)	1.391
10	LOQ (µg/mL)	4.214

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. The λ max for Cefoxitin sodium was determined in 0.1 M NaOH at 231 nm. At selected λmax and in its selected solvent system the drug showed a linear relationship (with correlation coefficient of 0.999) in the concentration range of 10 – 50 µg/mL. The linear regression equation for Cefoxitin sodium obtained was Y = 0.026X – 0.167. The data were shown in Table 3.

Linearity of regression equation was demonstrated from the high correlation coefficient value and very low values of intercept. Hence it was suggested that the

calibration line of Cefoxitin sodium in selected solvent system did not deviate from the origin and its values were within the acceptable range. Linearity curve for Cefoxitin sodium were shown in the Fig 3.

Table 3: Linearity table for Cefoxitin sodium.

Sr. No	Concentration (µg/mL)	Absorbance
1	10	0.099
2	15	0.217
3	20	0.35
4	25	0.476
5	30	0.61
6	35	0.741
7	40	0.879
8	45	1.01
9	50	1.12

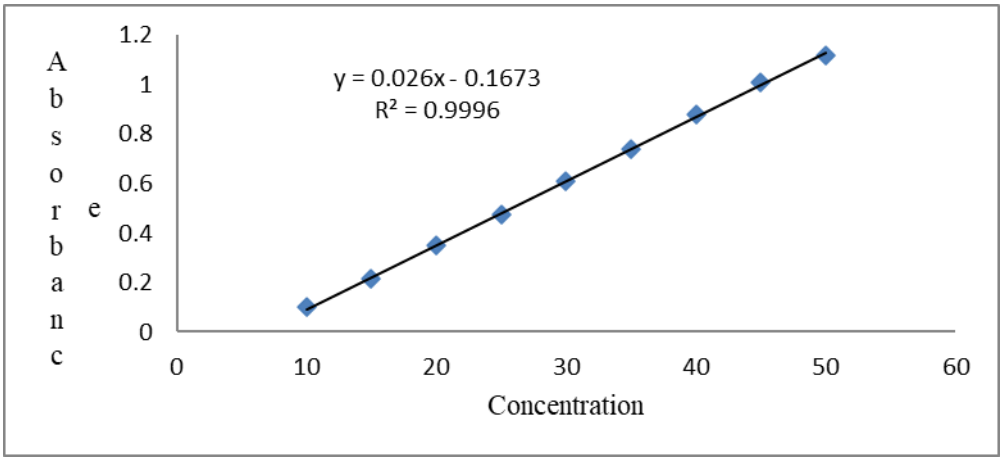

Fig. 3: Linearity graph for Cefoxitin sodium.

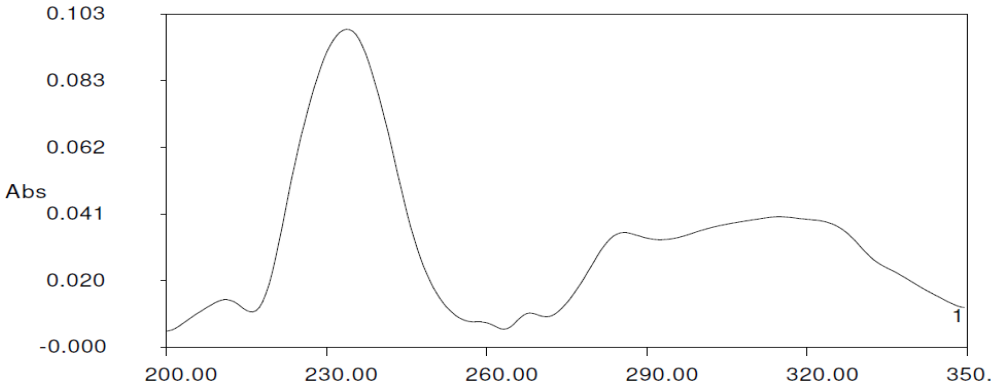

Fig. 4: UV spectrum for 10µg/mL of Cefoxitin sodium.

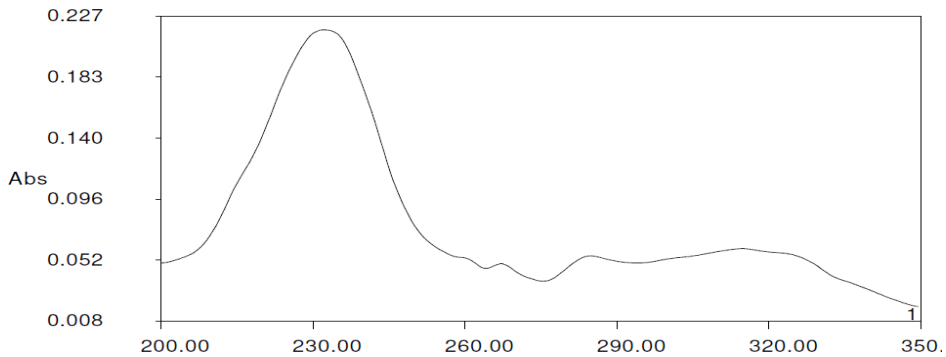

Fig. 5: UV spectrum for 15µg/mL of Cefoxitin sodium.

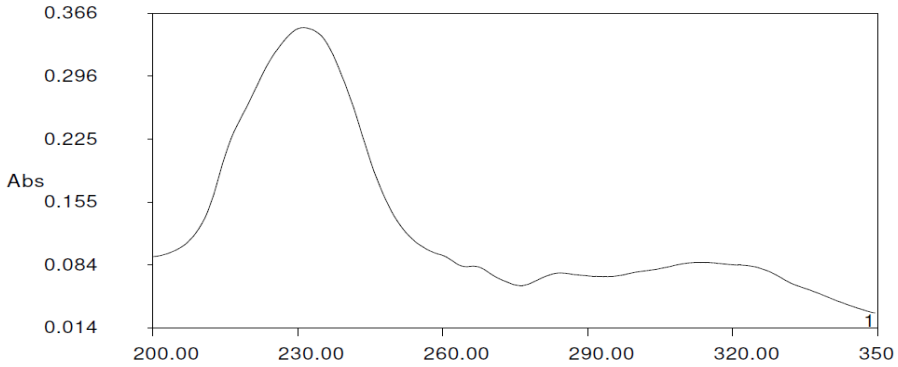

Fig. 6: UV spectrum for 20µg/mL of Cefoxitin sodium.

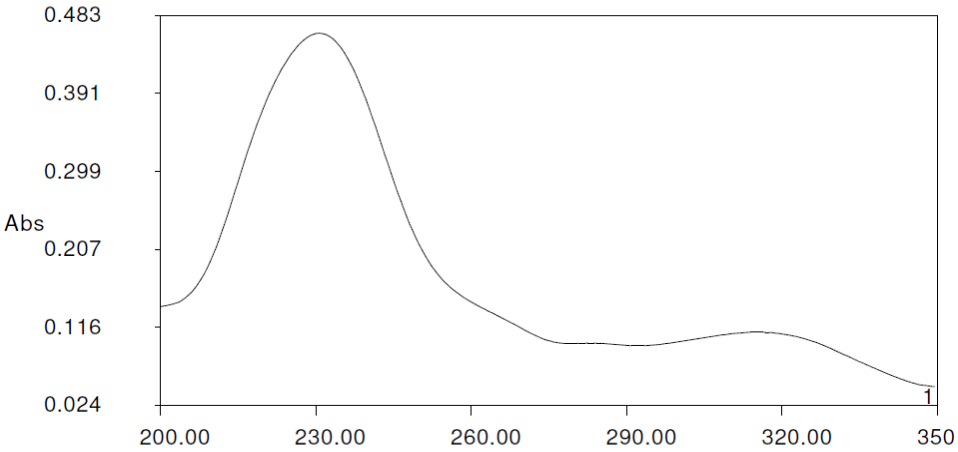

Fig. 7: UV spectrum for 25µg/mL of Cefoxitin sodium.

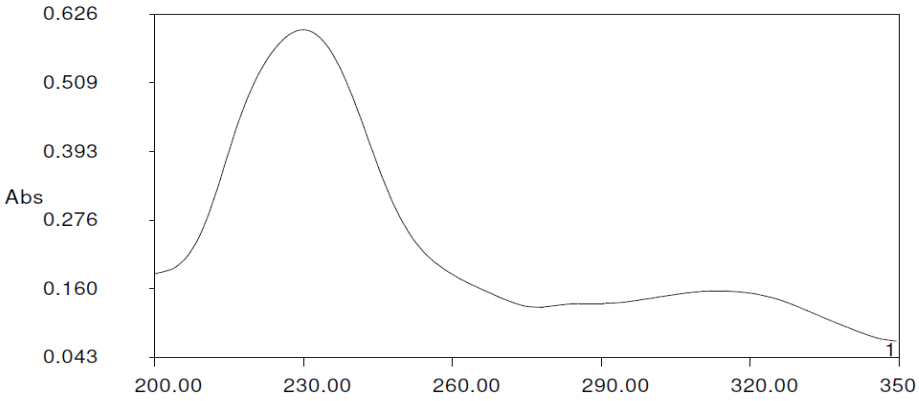

Fig. 8: UV spectrum for 30µg/mL of Cefoxitin sodium.

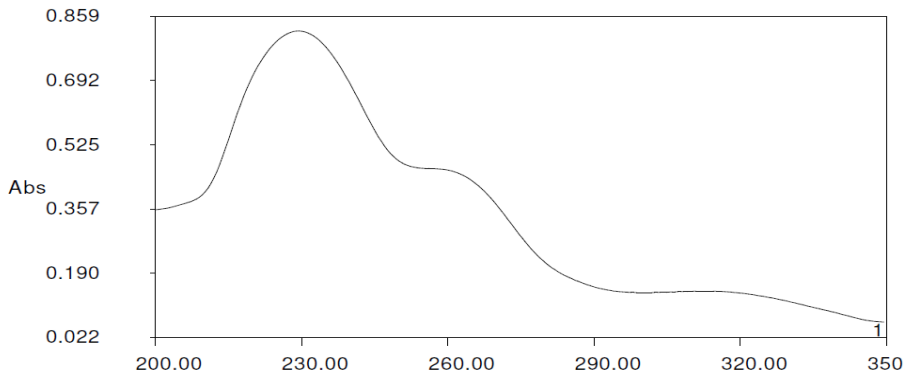

Fig. 9: UV spectrum for 35µg/mL of Cefoxitin sodium.

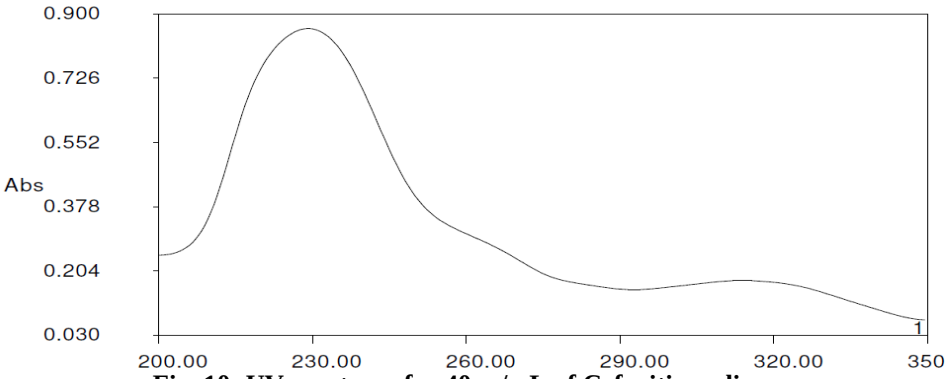

Fig. 10: UV spectrum for 40µg/mL of Cefoxitin sodium.

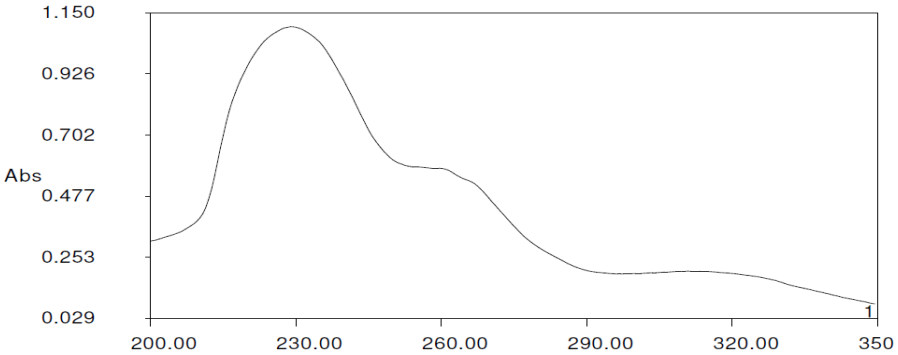

Fig. 11: UV spectrum for 45µg/mL of Cefoxitin sodium.

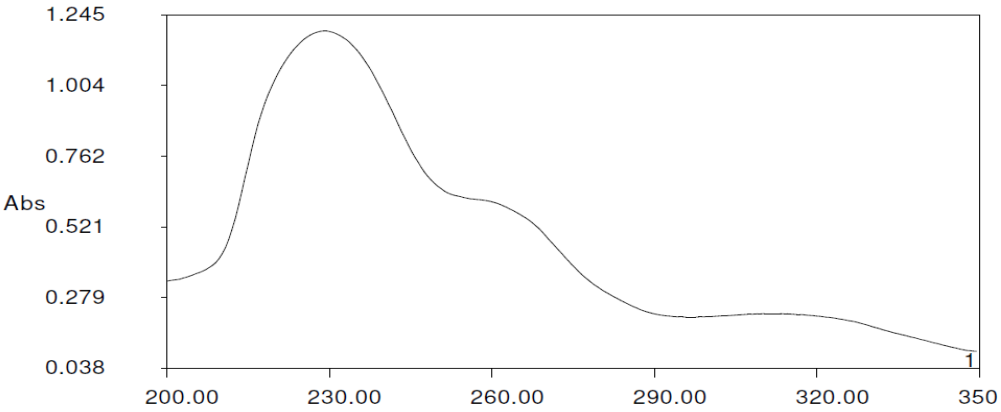

Fig. 12: UV spectrum for 50µg/mL of Cefoxitin sodium.

Accuracy

Accuracy of the proposed method was studied by analyzing the powder for injection formulation in three different concentration levels like 10%, 20% and 30 % under similar condition of the above procedure. The percentage recoveries of the three concentrations were found to be close to 100%. The results were tabulated in Table 4.

Table 4: Accuracy.

S.NO	Levels of std drug added (%)	Label claim of Cefoxitin sodium (mg)	Percentage recovery*	%RSD
1	10 %	1000	98.71	0.270
2	20 %	1000	99.52	0.306
3	30 %	1000	99.36	0.309

(*n=3)

Precision

To validate the precision ability of the proposed method, 20µg/mL concentration of Cefoxitin sodium sample was prepared. Six replicates on the same day (*interday* precision) and on six consecutive days (*Interday* precision) were carried out. The very low % relative standard deviation (% RSD) values for *intraday* and

interday indicated that the precision of the method was good and data's were summarized in Table 5.

Table 5: *Intra* and *Interday* precision for Cefoxitin sodium assay in Powder for injection dosage form.

Concentration	*Interday* precision			*Intraday* precision		
	Duration	% Content	% RSD	Day	% Content	% RSD
20µg/mL	9.0 am	99.34	0.736	1	98.29	0.511
	11.05 am	99.13		2	98.97	
	1.03 pm	99.51		3	99.71	
	3.10 pm	98.7		4	99.11	
	5.05 pm	97.20		5	98.47	
	7.11 pm	97.05		6	99.05	

SUMMARY AND CONCLUSION

A rapid, cheap, reliable and simple spectrophotometric method for the quantitive determination of Cefoxitin in powder for injection dosage forms was developed. The proposed method obeys Beer's law limit in the range of 10 – 50 µg/mL with an apparent molar absorptivity of 8.451 x 103 L mol-1 cm-1. Linear regression of absorbance on concentration gave the equation Y= 0.026X – 0.167, with a correlation co-efficient of R2 = 0.999. The detection wavelength was found at 231 nm. LOD and LOQ were found to be 1.39 µg/mL and 4.21 µg/mL for Cefoxitin sodium respectively. The accuracy of the method was proved by performing recovery studies which were carried out by analyzing the formulation with three different levels like 10%, 20% and 30%. A value closer to 100% indicates that the proposed method is accurate for the analysis. Thus, it was concluded that the above method was simple, precise and easy to perform and require short time to analyze the drug in the commercial formulations.

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